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HUMAN CYTOMEGALOVIRUS (CMV) AND INDIRECT IMMUNOFLUORESCENCE IN DISSEMINATED LUPUS ERYTHEMATOSUS

Serge Montplaisir and Bernard Martineau

Translation of "Virus cytomegalique (VCM) humain et immunofluorescence indirecte dans le lupus erythemateux dissemine (LED)," Canadian Journal of Microbiology, Volume 20, Number 3, 1974, pp. 418-420.

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16. Abstract

The authors investigate misleading results of test for cytomegalovirus infection and find that they may be conditioned by previous occurrence of disseminated Lupus erythematosus.

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HUMAN CYTOMEGALOVIRUS (CMV) AND INDIRECT IMMUNOFLUORESCENCE IN DISSEMINATED LUPUS ERYTHEMATOSUS[1,2,3]

Serge Montplaisir[4] and Bernard Martineau[5]

Research on anti-CMV antibodies by the indirect immunofluorescence method /418* has become a routine technique accelerating the serological diagnosis of infections caused by this virus [3,7].

The search for these antibodies in patients treated for DLE has produced positive reactions in cells infected by CMV, despite the absence of anti-CMV antibodies at the time of the complement fixation. It is very probable that these false positive results are caused by the presence of antinuclear antibodies reacting with the nuclear elements of fibroblasts and possibly with the antigenic matrix of the CMV.

The sera were taken from nine patients treated for DLE independently of the disease course and therapy used. The sera were preserved at -20°C and inactivated at 56°C for 30 minutes before each test. The techniques of fixing the complement, of culturing the fibroblasts and of infecting cells with CMV (strain AD169) have already been reported [6, 9, 10]. The fibroblastic cells were cultivated on lamellae in Leighton tubes. The culture media contained gammaglobulinemic fetal veal serum. When the cell covering had the characteristic inclusions, the lamellae were fixed in acetone at -20°C for 15 minutes, then preserved at -70°C.

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Indirect Immunofluorescence

The sera were diluted 1:5 with phosphate tampon (PBS), and then spread on the lamellae with a curved Pasteur pipette. The lamellae were incubated in a humid environment for 30 minutes and then washed in the PBS for 10 minutes. A /419
drop of human anti-IgG rabbit serum, conjugated with fluorescein was spread on each lamella and held in contact for 45 minutes. After a final 10 minute rinse in the PBS, the lamellae were fixed on a film with the help of tamponned glycerol. Each serum was tested for normal and infected fibroblasts. In each case the specificity of the reaction was verified by preliminary blocking of the fluorescence with a human, non cojugated, anti-IgG rabbit serum. The antinuclear antibodies had been determined with the same method using rat liver sections.

The complement fixation reaction allowed us to evaluate the immunization condition of this group of patients with regard to CMV. A number of epidemiological studies [2, 4], including the one carried out in our environment [9], has clearly demonstrated that these antibodies persist in their host at a titer $\leqslant$ 1:16. In the group study the titer was $\geqslant$ 1:16 in seven cases and negative in two cases (see Table 1). These results clearly confirmed the opportunistic role of the CMV, taking advantage of an area immunologically weakened by the preexistent disease and by the immunosupressive therapy.

In the indirect immunofluorescence test the titer of the antinuclear antibodies varied from 1:20 to 1:640 on rat liver sections, and all of the sera reacted with the normal and infected fibroblasts (see Table 1). In fact several studies have shown that the antinuclear antibodies constitute a very heterogeneous group capable of reacting with different antigenic patterns found in most of the nuclei. The presence of these antibodies prevent us from correctly interpreting the results obtained with immunofluorescence, and may be the cause of the "false positive" reactions, such as already reported for syphilis [5] and toxoplasmosis [1].

When the sera were positive at complement fixation (Cases 1-7), determination of the antibodies by immunofluorescence could no longer be interpreted as the only statement of a specific reaction between the anti-CMV antibodies and the viral antigens. When the sera were negative at complement fixation (Cases 8, 10), the results was a positive reaction to immunofluorescence giving a picture similar

to that found during infections caused by CMV. Such results should prompt us routinely to check normal cells and to absorb the serum at the beginning, either with ADN or on normal fibroblasts, when the antinuclear antibodies are present. In addition, since these two methods do not detect the same antibodies, it is theoretically possible that even in the absence of antinuclear antibodies a serum may give a positive reaction to immunofluorescence and a negative one to the complement fixation during infection by this virus.

Table 1. Serology of Cytomegalovirus (CMV) and Antinuclear Antibodies (ANA) in Nine Cases of Disseminated Lupus Erythematus (DLE).

Clinical Diagnosis		VCM		AAN[b]			
				Liver		Fibroblasts	
		FC[a]	IF[b]	Titer	Morphology	Test	Morphology
Case 1	LED	1/32	++++	1/20	Homogeneous	++++	Homogeneous
Case 2	LED	1/16	+++	1/40	Homogeneous	++++	Homogeneous
Case 3	LED	1/32	++++	1/80	Homogeneous	++++	Homogeneous
Case 4	LED	1/64	++++	1/20	Homogeneous	++++	Reticulated
Case 5	LED	1/64	++++	1/40	Reticulated	++++	Homogeneous
Case 6	LED	1/16	++++	1/640	Homogeneous + Nucleolar	++++	Perinuclear
Case 7	LED	1/64	++++	1/160	"Composit"++	++++	Homogeneous
Case 8	LED	0	+++	1/20	Nucleolar	+++	Homogeneous
Case 9	LED	0	++++	1/160	Reticulated	++++	Perinuclear
Case 10[d]	VCM	1/128	++++	0	Homogeneous	0	

[a] FC, complement fixation (0:≤1:4).

[b] Indirect immunofluorescence (IgG).

[c] Homogeneous coloration more intense at periphery.

[d] Mother of a newborn infant with the disease of cytomegalic inclusions.

Of the nine sera studied, six show a type of morphology different for rat liver sections and normal fibroblasts (see Table 1). This variation may be explained by a certain number of factors. The method of cell fixation, with alcohol for rat liver sections and acetone for fibroblasts, the effects produced by culturing the fibroblasts, the origin of the cells, different sera dilutions [8], are all variants capable of partially explaining the morphological modifications of the fluorescence. /420

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